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FINAL REPORT

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## NASA Final Report

**Summary:** A detailed investigation using electron microscopy was conducted which examined the conditions of materials used in the construction of stable, high performance microbore LC columns. Small details proved to be important. The effects of temperature on the elution of several homologous series used as probe compounds was examined in reverse phase systems. They showed that accessible temperature changes provide roughly half the increase in solvent strength that would be obtained going from a 100% aqueous to a 100% organic mobile phase, which is sufficient to warrant their use in many analyses requiring the use of gradients. Air circulation temperature control systems provide the easiest means of obtaining rapid, wide range changes in column temperature. However, slow heat transfer from the gas leads to thermal nonuniformity in the column and a decrease in resolution as the temperature program progresses. Liquid temperature control systems, because of their more rapid heat transfer, must be used if resolution during the temperature program is to be maintained. The stability of silica based reversed phase LC packings limits the maximum column temperature to 160 C. Column failure apparently results from the dissolution of the silica backbone of the sorbent and the formation of interior voids. Polymer based reversed phase packings show greater thermal stability at high operating temperatures, but trace levels of styrene monomer become entrained in the mobile phase, which interferes with UV detection.

## Introduction

A major goal for this grant was the development of a microbore LC/MS instrument. However, the lack of an available mass spectrometer for this purpose made this final realization unattainable. However, in lieu of a mass spectrometer, a great deal has been learned about the microbore LC portion of this instrument, which will undoubtedly be of great lasting value to future researchers in this field. The following is a condensation of work that is covered in more detail in the Dissertation "Microbore LC Methodology and Temperature programmed Microbore HPLC" (1).

## Physical Requirements

Initial commercial microbore columns were plagued by poor performance and short column lifetimes. Direct scaledown of conventional LC column packing methodology encountered several problems. These could be attributed mainly to a lack of attention to detail. The termination of microbore LC columns have a much smaller cross-sectional area than conventional LC columns, and special care must be taken not to restrict the mobile phase flow by crushing the inlet and outlet frits which contain the column packing. Small plastic surrounded frits were required to isolate these filters from the compressional forces occurring during column assembly (See figure 1).

Examination by electron microscopy of the interior walls of 1/16" tubing used to pack microbore columns (Figure 2) showed a pitted, highly irregular surface with short term variations of 20 microns in surface height. Columns packed with this tubing developed half the theoretical number of plates and typically failed through the formation of interior voids. A column polishing procedure was developed which removed these irregularities (Figure 3). Columns packed with this polished tubing typically delivered 100% of the resolution predicted by theory. Glass-lined stainless steel tubing was also used to pack microbore columns. These provided high performance columns as well. This was attributed to their smooth interior surface.

## Temperature Programmed LC

The magnitude of the change brought about by variations in column temperature was not clearly defined in the literature. Snyder (2) discounted temperature as a means to decrease analysis time and sharpen peaks in favor of changing the mobile phase composition during a run. Many authors note the necessity of maintaining a constant column temperature for obtaining reproducible retention times. Few gave guidelines for predicting the magnitude of temperature effects. They lacked a format for evaluating and comparing changes in temperature and mobile phase composition. Homologous series, that is, groups of compounds

which differ only in the number of repeating structural units in a side chain, show an internal consistency in elution which provides such a structured format for evaluating both temperature and mobile phase gradients. For alkyl homologs, the methylene selectivity provides an estimate of mobile phase strength, which is independent of the base group chosen for the series. Plotting the logarithm of the capacity factor  $k'$  ( $k' = t_r - t_0/t_0$ ) vs the homolog number gives a slope ( $\log \alpha$ ) that indicates the strength of the eluting system. Figure 4 shows a comparison of mobile phase strength and temperature using the "log  $\alpha$ " concept. It shows that a 70/30 methanol/water mobile phase at 0 C has the same strength ( $\log \alpha$ ) as a 60/40 methanol/water mobile phase at 75 C. While the lines in figure 4 are not strictly parallel, extrapolation based on a 3.75 C per each 1% increase in mobile phase composition shows that a span of 180 C would be equal to a 48% change in mobile phase composition. Thus, temperature provides roughly half the magnitude of change in elution strength as mobile phase changes using a single organic modifier with water. Since many gradient analyses require less span in solvent strength than this to show improvements in analysis speed and sensitivity, temperature gradients can be substituted for solvent gradients to provide the required improvements in chromatographic performance without the need to change the mobile phase composition. Changes in mobile phase composition in the small volumes used with microbore columns has been particularly difficult to achieve with any reproducibility. Temperature is an easier variable to control accurately through electronic feedback devices.

#### Temperature Control Systems for LC

Two methods of controlling the columns temperature were employed in this study. One surrounded the column with a liquid which was circulated through a commercial constant temperature bath (Figure 5). The heating coils and refrigeration unit in the bath provided convenient access to constant temperatures ranging from -20 C to +105 C. The 4 liter liquid volume was beneficial in maintaining these temperatures constant. However, when used to produce temperature programs, the 800 W heater was only capable of delivering a 0.75 C per minute temperature rise. This would be equivalent to a 1% per 5 minute rise in organic modifier content using conventional solvent gradients, which is much too slow to be of any value chromatographically.

A faster method for heating the column was constructed out of a gas chromatographs' temperature control module which was used to regulate the power input to the heating coils of a heat gun. An enclosure around the microbore column contained the hot air and a temperature sensor mounted on the column provided the necessary signals to the temperature control module (Figure 6). Very high programming rates (up to 50 C/min) were possible. An example of the effect of a 5 C/min temperature gradient on the elution of a series of homologs is shown in figure 7. A dramatic improvement in peak shape and a reduction in total analysis time was seen for the late eluting members of the series.

A study of the number of theoretical plates generated at different temperatures and flow rates was conducted to see if the decrease in viscosity of the mobile phase and the increase in diffusion coefficient of the sample at higher operating temperatures would translate into shorter analyses times with greater resolution. Both temperature control systems (air and liquid) were used, and the results appear in figures 8 and 9. As can be seen in figure 8, a dramatic drop in the number of theoretical plates is observed as the flow rate increased for the air temperature control system, but the number of theoretical plates for the liquid temperature control system (figure 9) remains the same and even rises slightly with flow rate. These results suggest that the temperature of the entering mobile phase in the column surrounded by hot air does not rise as quickly as it does when the column surrounded by a heated liquid. The cooler zone at the head of the air controlled column is longer than that for the liquid controlled column. The more strongly retained species are held in the cooler portions of the column and spread out in time. The larger cool portion in the air controlled column translates to wider peak widths and poorer resolution. To maintain the resolution in temperature programmed LC, a liquid temperature control system must be employed. A much smaller liquid heater than the constant temperature bath could be designed for this purpose. A closed, pressurized system would permit operation to high temperatures.

### Column Stability

Reversed phase columns are usually made from porous silica which has been chemically bonded layer of C-18 wax. This material is hydrolytically stable at room temperatures but in the presence of oxygen or strong acids, it may cleave from the surface of the silica at high temperatures. The initial chemical bonding reaction involves mixing chloroalkylsilanes with the silica, reacting them under reflux of solvents like toluene or xylene, washing the final product with toluene (to remove excess chloroalkylsilanes) and then quenching the reaction with methanol. In the process, some unbound alkylsilanes are produced which are insoluble in the wash solvents. The first time a column packed with this material is heated, this unbound material melts and is carried off the column where it can plug thin transfer lines and even the detector cell (Figure 10). Once this material is removed, the column may be used in temperature programs at temperatures as high as 160 C. After a few weeks of use, the columns performance begins to degrade. Void formation has been observed in columns used over prolonged periods with temperature programming. This may result from dissolution of the silica backbone of the reversed phase packing.

Recently, polymer-based reversed phase packings have become available which are made from cross-linked styrene-divinyl benzene. Since these materials do not contain wax or a silica backbone, they were studied as alternative sorbents for temperature programmed LC. Columns packed from Polymer Labs 10 um material gave columns with moderate performance.

No optimization of the packing procedure was attempted. During the temperature programmed run, the column gave the expected reduction in backpressure resulting from the decrease in solvent viscosity, without any rises due to transfer line blockage. However, the detector baseline rose considerably and with a 80/20 methanol/water mobile phase went off scale at 115 C. Reducing the mobile phase strength to 70/30 allowed operation to 125 C and at 60/40 the upper limit was 135 C. Apparently, trace levels of the monomer bleeds off the packing and its level increases at increasing temperatures. Styrene and divinyl benzene possess formidable extinction coefficients in the UV ( $>10,000$ ) and even at trace levels severely interfere with UV detectors. An advantage of silica based reverse phase materials is that the substance which bleeds from them does not possess any noticeable absorption in the UV. Reverse phase packings based on graphite might show greater promise for use in temperature programmed LC, but such material is still experimental (3).

#### References

1. Bowermaster, J., "Microbore Methodology and Temperature Programmed Microbore HPLC", Dissertation, Virginia Polytechnic Institute and State University, 1985.
2. Snyder, L. R.: J. Chromatogr. Sci., 7 (1969) 692-706.
3. Colin, H.; Eon, C.; Guiochon, G.: J. Chromatogr., 122 (1976) 223-242.

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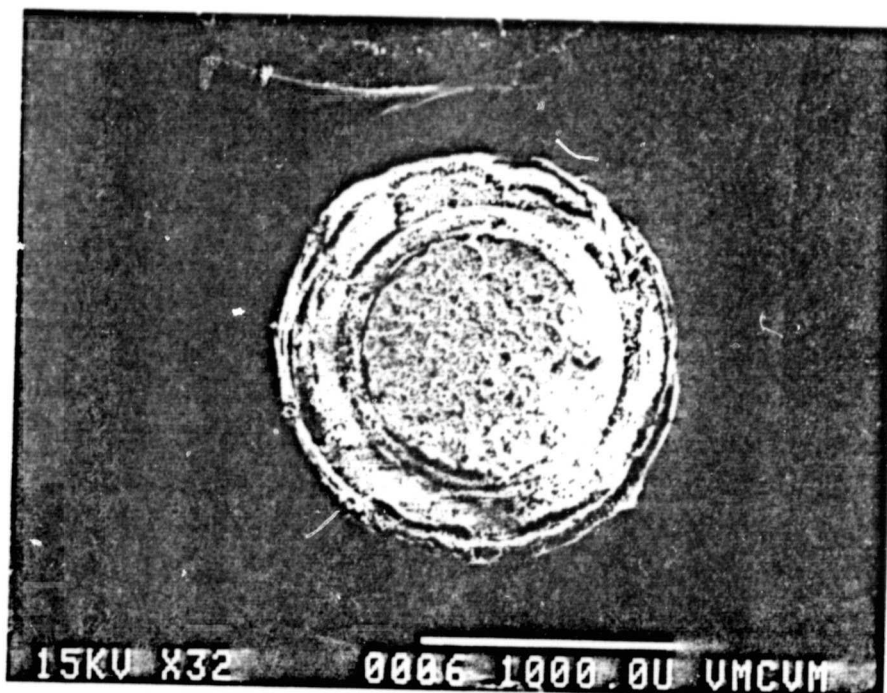
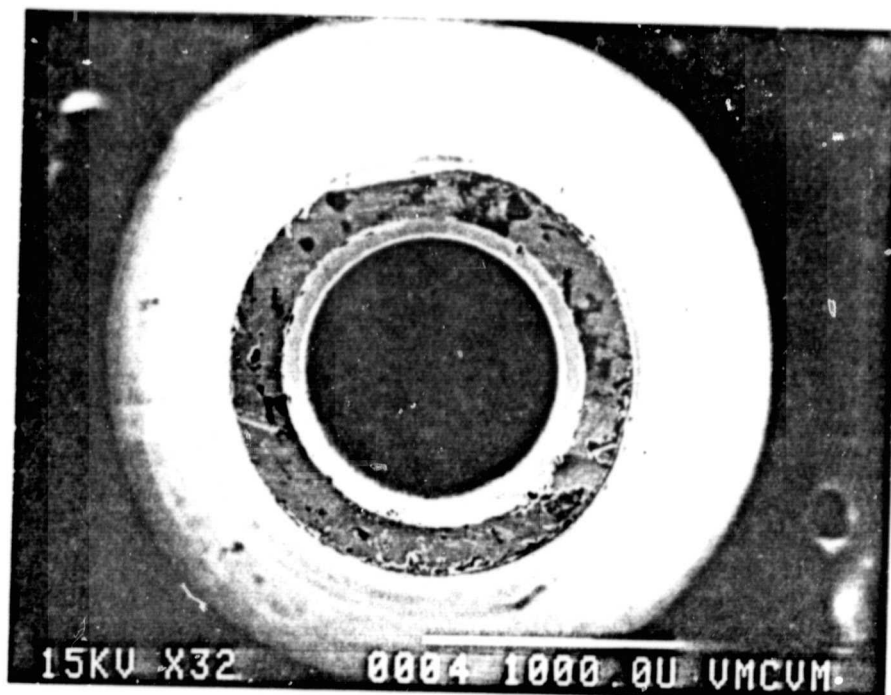


Figure 1. Beveled microbore column end and the seal made with the Kel-F® surrounded frit.



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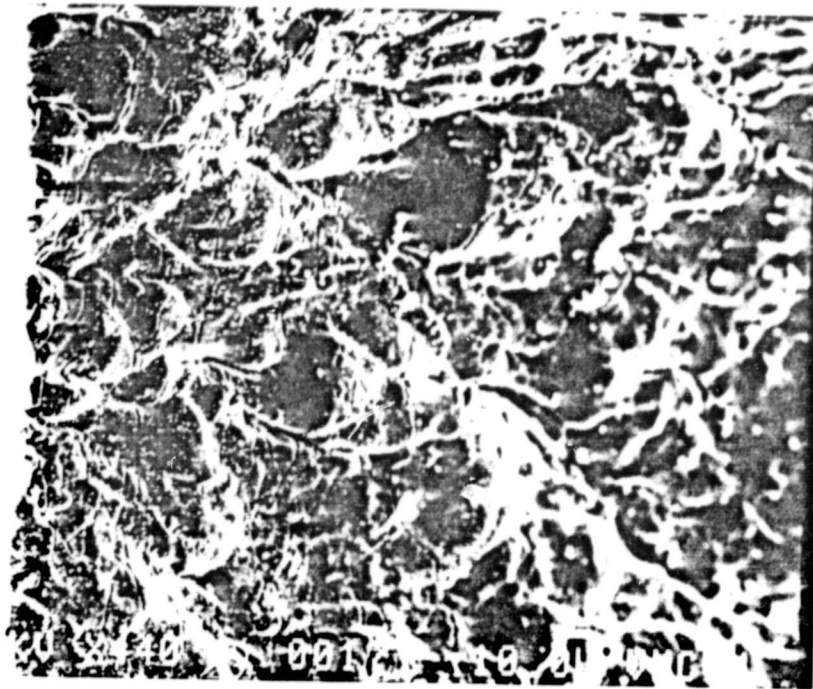
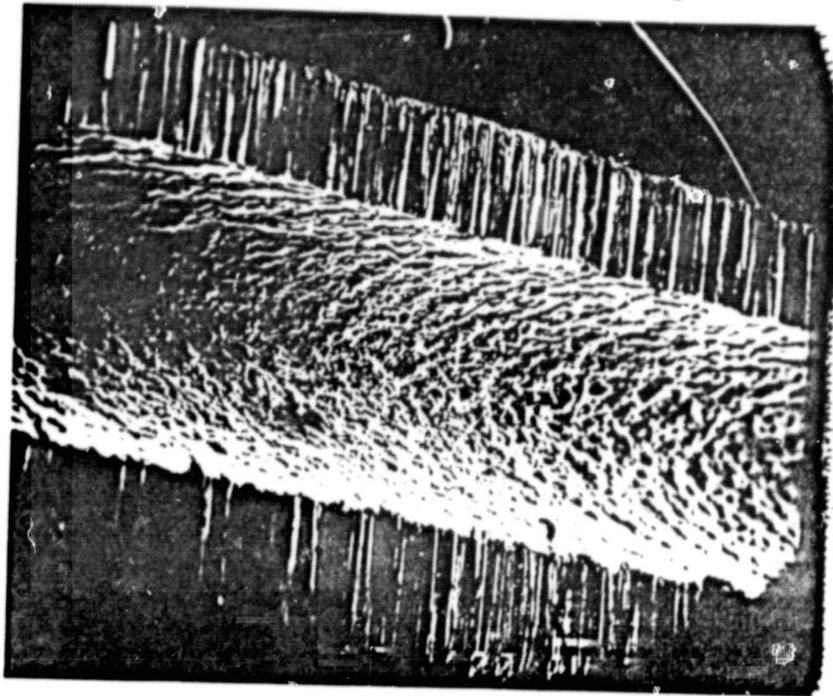


Figure 2. Inside surface of commercial 1/16" ID stainless steel tubing.

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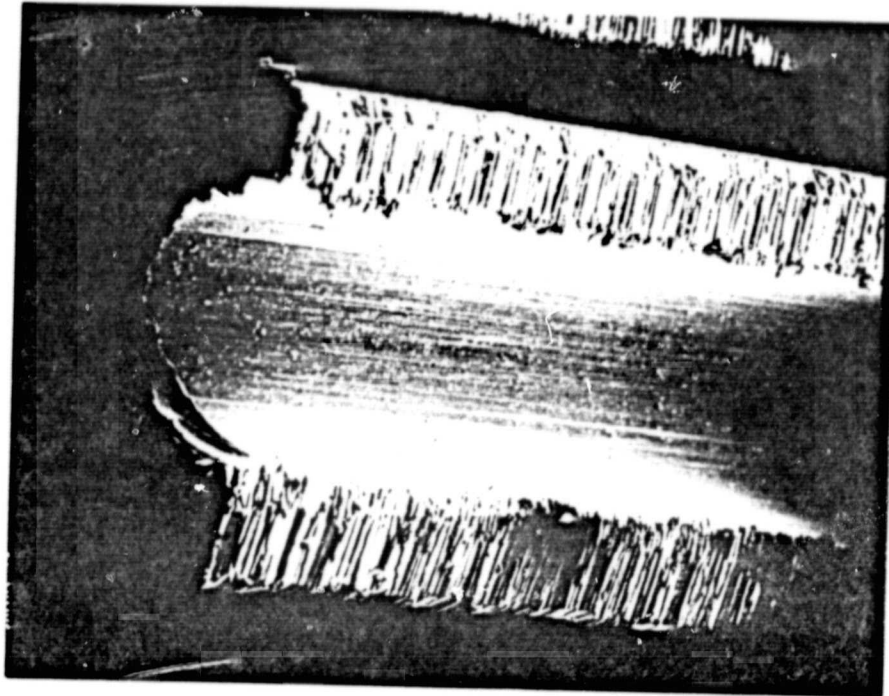


Figure 3. Inside tubing finish after polishing.

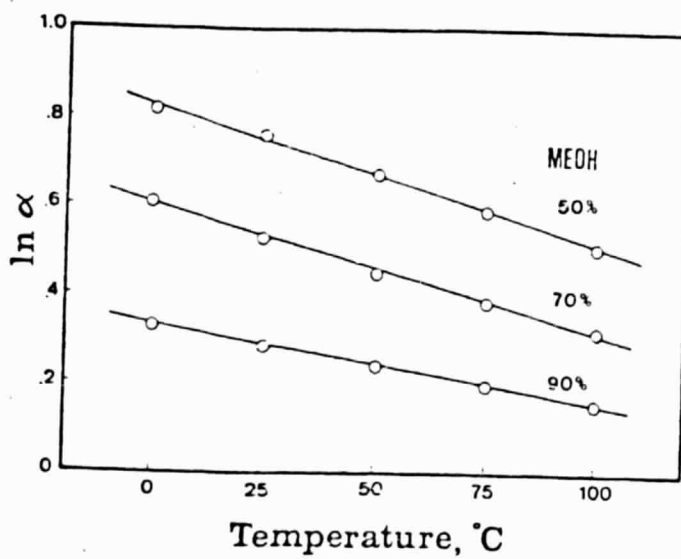


Figure 4.  $\ln \alpha$  versus temperature.

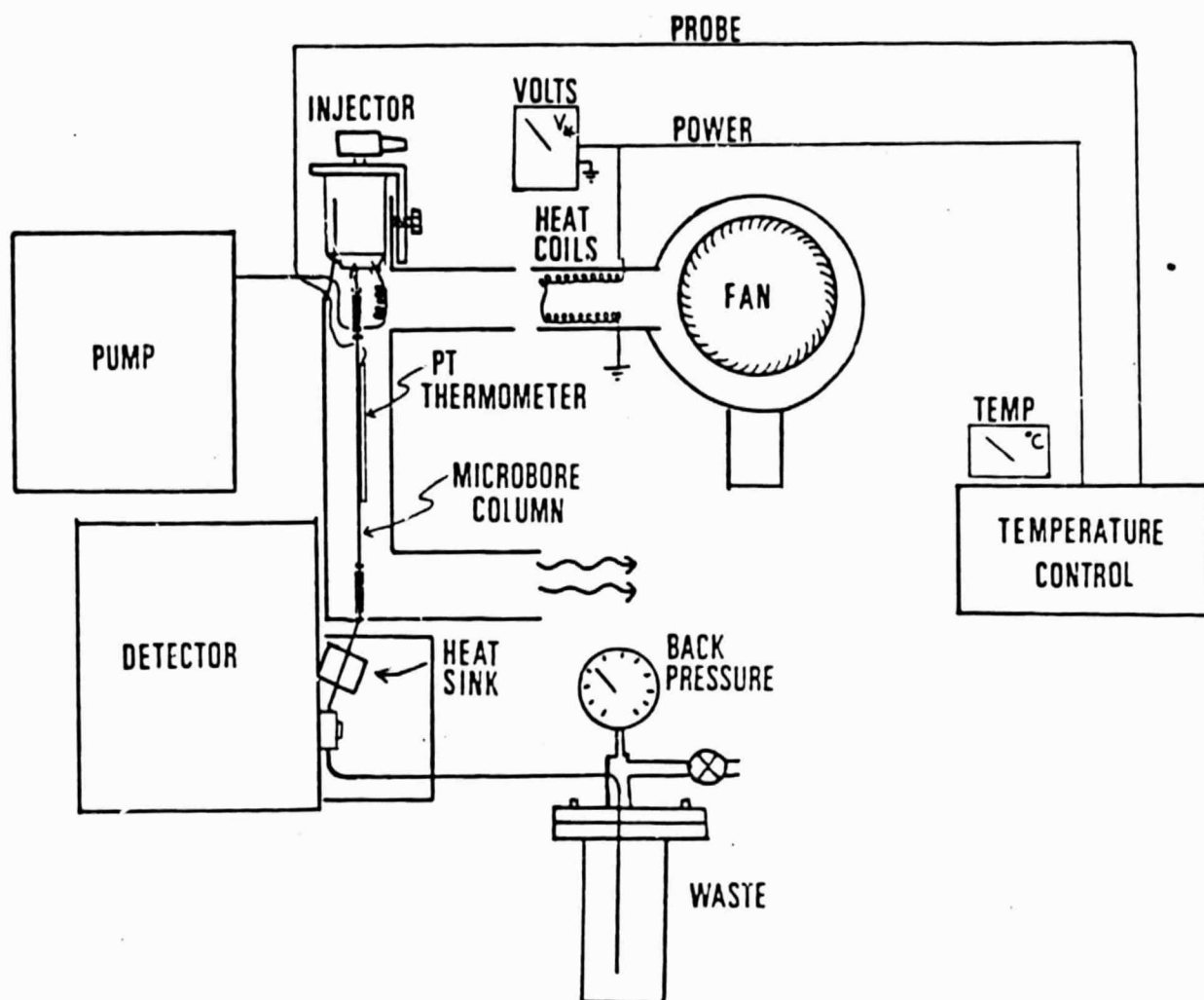


Figure 6. Heated air column temperature control system.

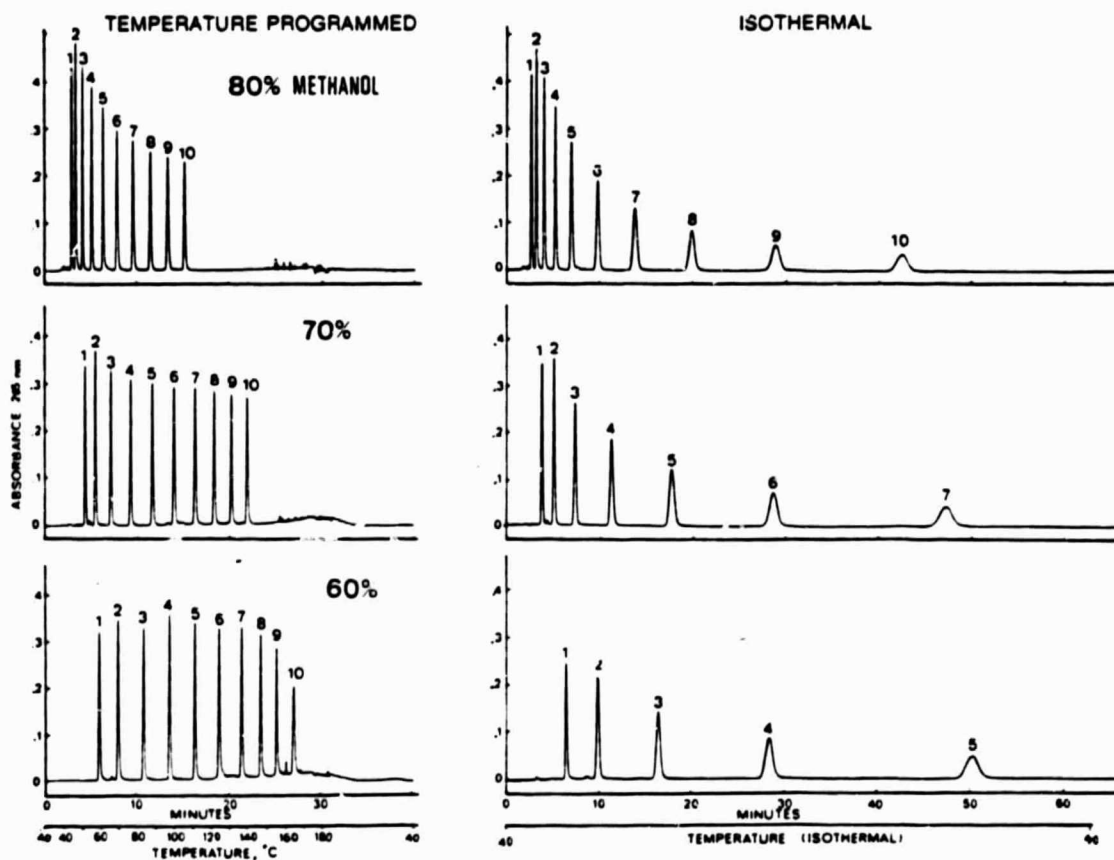


Figure 7. Temperature programmed microbore HPLC.

Column - 25 cm x 1 mm ID  
 Sorbent - LiChrosorb RP-18, 10  $\mu$ m  
 Sample -  $C_1$ - $C_{10}$  Alkyl Benzenes

Left - Temperature Programmed at 5° C./min.  
 from 40° C. to 180° C.  
 Right - Isothermal at 40° C.

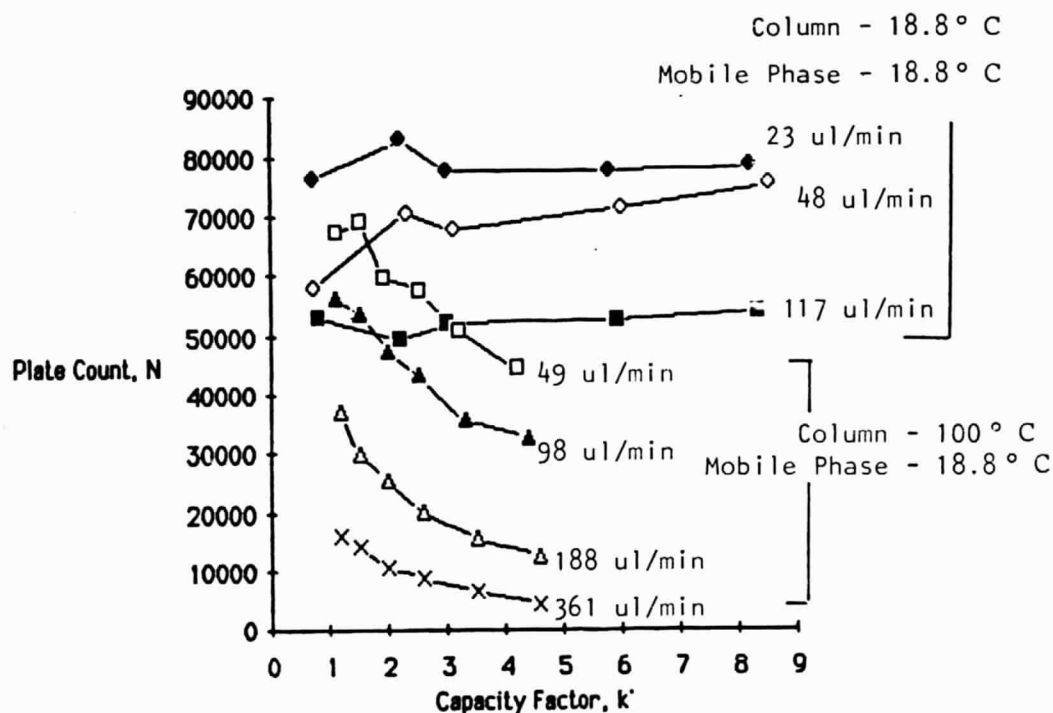


Figure 8. Observed column efficiency as a function of the flow rate, the capacity factor, and temperature of the column and the mobile phase using an air heating system.

Column - 25 cm x 1 mm ID  
Sorbent - LiChrosorb RP-18, 10  $\mu$ m  
Solvent - 90/10 MeOH/H<sub>2</sub>O  
Sample - 18.8° - C<sub>1</sub>-C<sub>5</sub> Alkyl Benzenes  
100° - C<sub>1</sub>-C<sub>6</sub> Alkyl Benzenes

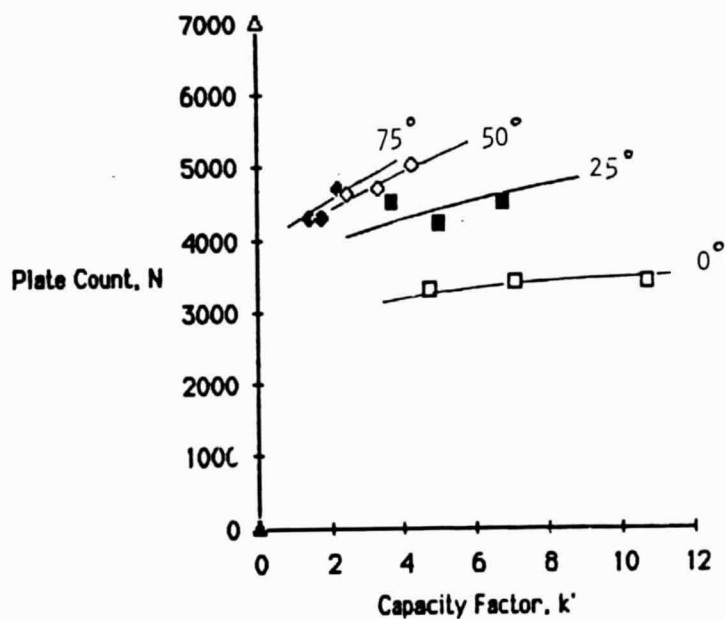


Figure 9. Column efficiency versus temperature for a water jacketed microbore column.

Column - 25 cm x 1 mm ID  
 Sorbent - LiChrosorb RP-18, 10  $\mu$ m  
 Solvent - 90/10 MeOH/H<sub>2</sub>O  
 Flow Rate - 100  $\mu$ l/min.  
 Sample - C<sub>8</sub>-C<sub>10</sub> Alkyl Benzenes

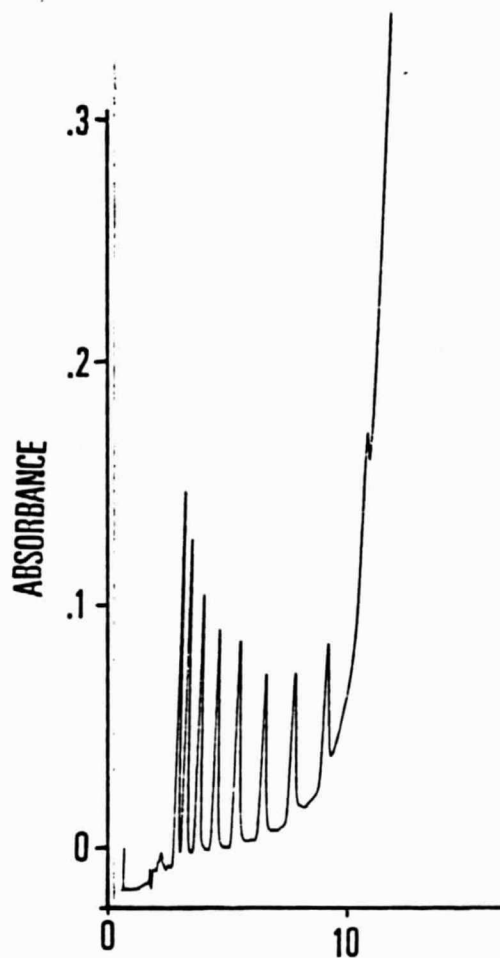


Figure 10. Temperature programmed chromatogram showing the effects of detector cell blockage by wax solidifying inside the detector cell.

Column - 25 cm x 1 mm ID  
 Sorbent - LiChrosorb RP-18, 10  $\mu$ m  
 Mobile Phase - 90/10 MeOH/H<sub>2</sub>O  
 Flow Rate - 100  $\mu$ l/min.  
 Initial Temp. - 30° C.  
 Program Rate - 5° C./min.  
 Sample - C<sub>1</sub>-C<sub>9</sub> Alkyl Benzenes